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Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: Vaccine

❷ Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:
Corporate Name: SmithKline Beecham Biologicals s.a.

Country (*and State of incorporation, if appropriate*): Belgium

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please given the following details:

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❹ Reference number

4. Agent's or applicant's reference number *(if applicable)* RE/RH/B45159

❺ Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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Yes ☐ No ☒ ➲ *go to 6*

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Yes ☐ No ☒ ➲ ***A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).).***

❽ *Please supply duplicates of claim(s), abstract, description and drawings).*

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8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77 [–]

Claim(s) 4 Description 18

Abstract [ ] Drawing(s) [ ]

8b Which of the following documents also accompanies the application?

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Vaccines

The present invention relates to improved vaccines, adjuvant systems, and processes for the preparation of such vaccines and adjuvant systems. In particular, the vaccines and adjuvant systems of the present invention comprise metallic salts, and a saponin such as Quil A, or derivatives thereof, optionally further comprising monophosphoryl lipid A, or its derivative 3-O-deacylated monophosphoryl lipid A.

Aluminium salts are well known in the art as providing a safe excipient with adjuvant activity. The mechanism of action of these adjuvants are thought to include the formation of an antigen depot such that antigen may stay at the site of injection for up to 3 weeks after administration, and also the formation of antigen/metallic salt complexes which are more easily taken up by antigen presenting cells. In addition to aluminium, other metallic salts have been used to adsorb antigens, including salts of zinc, calcium, cerium, chromium, iron, and berilium. The hydroxide and phosphate salts of aluminium are the most common.

Vaccine formulations containing aluminium salts, antigen, and saponins are known in the art. Such formulations induced greater immune responses in comparison with those stimulated by aluminium salts and antigen alone. Formulation of these vaccine preparations have previously involved a specific manufacturing procedure, since it was believed that in order for optimal immune responses to occur, the antigen must be adsorbed onto the same aluminium salt particle as the immunostimulant. In this way when antigen is taken up by an antigen presenting cell, the co-adsorbed saponin exerts its stimulatory activity directly onto that same antigen presenting cell.

Aluminium based vaccine formulations wherein the antigen and the saponin, QS21, are adsorbed onto the same particle are described in WO 98/15287. In this document the vaccines are prepared by adsorbing QS21 (associated with cholesterol containing liposomes) onto preadsorbed antigen/alum complexes. Thus, both the antigen and the QS21 are adsorbed onto the same particle of metallic salt. The formulation of the QS21 into cholesterol containing liposome was found to have two

major functions in that the reactogenicity was quenched and also the binding capacity of the QS21 to the alum was greatly enhanced.

The formulation processes of the prior art provide for potent vaccines from an immunological point of view, however, they do contain several commercial disadvantages. In order for a vaccine to be suitable for human administration, the process must be uniform and be subject to Good Manufacturing Practice (GMP) control, and and Quality Control (QC). In some cases of combination vaccines, the processes of the prior art provide a vaccine wherein all of the antigen, or antigens, are adsorbed onto the same particle of metallic salt. The process is then complicated by the requirement for the QS21 to be adsorbed onto the same metallic particle. This may be particularly problematical in the case of combination vaccines containing multiple antigens (whose adsorption may be dependent on the affinity of each antigen to the particular metallic salt at a given pH). The processes of the prior art have problems, depending on which antigens are present, in reproducibility and vaccine QC. Furthermore, if anything undesired occurs with the QC of one particular antigen, or an occurrence which may result in the contamination of the vaccine, this may result in the waste all of the individual components, and not just the particular antigen in which the problem occurred. Moreover, in some circumstances combination vaccines may require the sequential addition of the antigens, such a process being hugely time consuming and expensive. The processes of the prior art may, therefore, be complex, difficult to control, and expensive.

Surprisingly, the present inventors have discovered that it is not necessary to adsorb antigen and the saponin onto the same particle. In contrast to the accepted thinking in the art, it has been found that good vaccines may be produced when antigen is adsorbed onto particular metallic salt particles which are discrete from those metallic salt particles which are associated with the saponin.

The improved process comprises the adsorption of saponin, onto a metallic salt particle, followed by the adsorption of the antigen onto another metallic salt particle, followed by the mixing of the discrete metallic particles to form a vaccine.

The present invention also provides for an adjuvant composition comprising a saponin, adsorbed onto a metallic salt particle, characterised in that the metallic salt particle is substantially free of antigen. Furthermore, vaccines are provided by the present invention and are characterised in that the saponin is adsorbed onto particles of metallic salt which are substantially free from other antigen, and in that the particles of metallic salt which are adsorbed to the antigen are substantially free of saponin.

Accordingly, the present invention provides an adjuvant formulation comprising saponin which has been adsorbed onto a particle of a metallic salt, characterised in the composition is substantially free of other antigen. Moreover, this adjuvant formulation is an intermediate which is required during the process of the present invention, for the manufacture of a vaccine. Accordingly there is provided a process for the manufacture of a vaccine comprising admixing the adjuvant composition of the invention with an antigen. Preferably, the antigen has been pre-adsorbed onto a metallic salt. Said metallic salt may be identical or similar to the metallic salt which is adsorbed onto the immunostimulant.

The present invention further provides for a vaccine composition comprising a saponin adsorbed onto a first particle of a metallic salt, and antigen adsorbed onto a metallic salt, characterised in that first and second particles of metallic salt are different.

Alternatively, vaccines which form part of the present invention comprise two major populations of complexes, a first complex comprising (a) a saponin adsorbed onto a metallic salt particle, characterised in that said metallic salt particle is substantially free of antigen; and a second complex comprising (b) antigen adsorbed onto a metallic salt particle. Also the vaccine composition can comprising two major populations of complexes, a first complex comprising (a) a saponin adsorbed onto a metallic salt particle, characterising in that said metallic salt particle is substantially free of antigen; and a second complex comprising (b) antigen adsorbed onto a

metallic salt particle, characterised in that said metallic salt particle is substantially free of saponin.

The metallic salts present in these two major populations of complexes may be identical or different. Furthermore, in the case of a combination vaccine, wherein a plurality of different antigens may be present, the second complex (described above) may comprise a plurality of antigens adsorbed onto different metallic particles.

The definition of substantially free, in relation to this invention, is where not more than 20% by mass of the total material capable of adsorbing to the particle of metallic salt is an other antigen, preferably not more than 10%, and most preferably not more than 5%. Alternatively, the substantially free, in relation to this invention, is where not more than 20% by mass of the total material capable of adsorbing to the particle of metallic salt is a saponin, preferably not more than 10%, and most preferably not more than 5%. Routine assays could be used to determine whether the antigen and immunostimulant, are adsorbed onto different discrete particles, including seperation of the vaccine into distinct fractions by free flow of the formulation within an electric field, or techniques such as sedimentation rate analysis which are particularly suited to non-particulate antigens, followed by assaying for the saponin or antigen in the fractions.

Also provided in the present invention is a kit comprising one container having saponin adsorbed onto a metallic salt; and a second container having antigen, preferably said antigen being adsorbed onto a metallic salt.

The process of the present invention is especially useful when commercial scale quantities of combination vaccines are required. Combination vaccines are single dose vaccines which contain more than one antigen from more than one pathogen. Such vaccines may reduce the number of vaccinations required to induce protection against many pathogens and diseases.

For example, if a vaccine comprises $AlOH_3$, QS21, and the antigens V, W, X, Y, Z, previous processes involve formulating the antigens and the QS21 onto the same particle of $AlOH_3$. Such prior art processes require that V, W, X, Y, Z are adsorbed onto the $AlOH_3$, followed by the addition of free QS21 onto each of the pre-adsorbed antigen complexes.

In contrast, the formulation process of the present invention antigens V, W, X, Y, Z are each individually adsorbed onto separate particles of QS21 in separate containers. QS21 is also adsorbed onto $AlOH_3$ in another container. The vaccine is then formed by the simple admixing of material taken from each of the separate containers. In this case the particles of $AlOH_3$ which is associated with the QS21 are discrete from the particles of $AlOH_3$ which are associated with the antigens.

Alternatively, the present invention provides a process of making a vaccine comprising a saponin, antigen and a metallic salt, comprising:

1. Adsorbing antigen to a first particle of metallic salt,
2. Adsorbing the saponin to a second particle of a metallic salt, and
3. admixing the products of steps 1 and 2 above.

The present invention provides for a process for the manufacture of vaccines which overcome the problems present in the prior art. Each individual antigen-metallic salt complex may be subject to GMP controls, and should any untoward contamination of a particular antigen-metallic salt preparation then the integrity of other antigens and saponin adjuvant will not be compromised. Surprisingly, and in contrast to the accepted thinking in the art, vaccines produced by the process of the present invention are as potent as those prepared using the process of the prior art.

Saponins are described in the Merck index (entry 8503, 12 Edition), as "*a type of glycoside widely distributed in plants. Each saponin consists of a sapogenin, which constitutes the aglucon moiety of the molecule, and a sugar. The sapogenin may be a sreroid or a triterpine and the sugar moiety may be glucose, galactose, a pentose, or a methyl pentose.*" Saponins are said to be bitter to taste and foam strongly when

shaken. Different saponins are widely known in the art including those derived from the the South American tree *Quilaja Saponaria Molina.*

Quil A is a saponin preparation isolated from *Quilaja Saponaria Molina* and was first described to have adjuvant activity by Dalsgaard *et al.* in 1974 ("Saponin adjuvants", Archiv. für die gesamte Virusforschung, Vol. 44, Springer Verlag, Berlin, p243-254). Purified fragments of Quil A have been isolated by HPLC which retain adjuvant activity without the toxicity associated with Quil A (EP 0 362 278), for example QS7 and QS21 (also known as QA7 and QA21). Particular formulations of QS21 have been described which are particularly preferred, these formulations further comprise a sterol (WO96/33739). QS21 is also described in Kensil *et al.* (1991. J. Immunology vol 146, 431-437).

The present invention relates to the particular formulation process and characteristics of the adjuvant, and thus can be utilised with a wide variety of antigens. The vaccines of the present invention can be used for priming and boosting doses, and used for the induction of immune responses to, and protection from infection mediated by, a wide variety of antigens. Also the present invention provides for a method of eliciting an immune response to an antigen comprising the use of a vaccine comprising a metallic salt, saponin, and antigen, wherein the saponin is adsorbed onto particles of metallic salt which are descrete from those metallic salt particles which are adsorbed to the antigen. Some of the pathogens and antigens are listed below.

Viral hepatitis, caused by the A, B, C, D, and E hepatitis viruses, is a very common viral illness. Via the B and C viruses, in particular, it is also responsible for many cases of liver cancer. Thus the development of effective vaccines is critical and, despite notable successes, is still an on-going task. A review on modern hepatitis vaccines, including a number of key references, may be found in the Lancet, May 12th 1990 at page 1142 ff (Prof A.L.W.F. Eddleston). See also 'Viral Hepatitis and Liver Disease' (Vyas, B.N., Dienstag, J.L., and Hoofnagle, J.H., eds, Grune and Stratton, Inc. (1984) and 'Viral Hepatitis and Liver Disease' (Proceedings of

the 1990 International Symposium, eds F.B. Hollinger, S.M. Lemon and H. Margolis, published by Williams and Wilkins).

As used herein the expression 'hepatitis B antigen' is used to refer to any antigenic material derived from a hepatitis B virus which may be used to induce immunity to the virus in humans.

Infection with hepatitis B virus (HBV) is a widespread problem but vaccines which can be used for mass immunisation are now available, for example the product 'Engerix-B' (SmithKline Beecham plc) which is obtained by genetic engineering techniques.

The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See. for example, Harford et al in Develop. Biol. Standard 54, page 125 (1983), Gregg et al in Biotechnology, 5, page 479 (1987), EP-A- 0 226 846, EP-A-0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen' or 'HBsAg' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et al, Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. In particular the HBsAg may comprise a polypeptide comprising an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L-protein of HBsAg relative to the open reading frame on a Hepatitis B virus of ad serotype (this polypeptide is referred to as L*; see EP 0 414 374). HBsAg within the scope of the invention may also include the preS1-preS2 -S polypeptide described in EP 0 198 474 (Endotronics) or analogues thereof such as those described in EP 0 304 578 (Mc Cormick and Jones). HBsAg as herein described can also refer to mutants, for example the 'escape mutant' described in WO 91/14703 or European Patent Application Publication Number 0 511 855 A1,

especially HBsAg wherein the amino acid substitution at position 145 is to arginine from glycine.

Normally the HBsAg will be in particle form. The particles may comprise for example S protein alone or may be composite particles, for example (L*,S) where L* is as defined above and S denotes the S-protein of HBsAg. The said particle is advantageously in the form in which it is expressed in yeast.

The component affording protection against Hepatitis A is preferably the product known as 'Havrix' (SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidiate Vaccines for Hepatitis A' by F.E. Andre, A. Hepburn and E.D'Hondt (1980), Prog. Med. Virol. Vol 37, pages 72-95 and the product monograph 'Havrix' published by SmithKline Beecham Biologicals (1991).

Thus, in a preferred embodiment of the present invention a combination vaccine comprising HBsAg and Hepatitis A antigen is provided. Also, provided by the present invention is a process for the production of a hepatitis A and B combination vaccine, and a product derived from that process.

Other combination vaccines are available on the market including the Infanrix™ range, made by SmithKline Beecham Biologicals. Such vaccines are based on a "core" combination of Diptheria toxin, Tetanus toxin, and *B. pertussis* antigens. This vaccine comprises a pertussis component (either killed whole cell *B. pertussis* or accellular *pertussis* which typically consists of two antigens - PT and FHA, and often 69kDa, optionally with one or both agglutinogen2 or agglutinogen 3). Such vaccines are often referred to as DTPw (whole cell) or DTPa (acellular).

Particular combination vaccines within the scope of the invention include:

Diptheria-Tetanus- Pertussis-Hepatitis B (DTP-HB)

Diptheria-Tetanus-Hepatitis B (DT-HB)

Hib-Hepatitis B

DTP-Hib-Hepatitis B

IPV (inactivated polio vaccine)- DTP-Hib-Hepatitis B

The pertussis component is suitably a whole cell pertussis vaccine or an acellular pertussis vaccine containing partially or highly purified antigens. The above combinations may optionally include a component which is protective against Hepatitis A. Preferably the Hepatitis A component is formalin HM-175 inactivated. Advantageously, the HM-175 is purified by treating the cultured HM-175 with trypsin, separating the intact virus from small protease digested protein by permeation chromatography and inactivating with formalin. Advantageously the Hepatitis B combination vaccine is a paediatric vaccine.

Other combination vaccines of the present invention are disclosed in GB 9805105.5 (SmithKline Beecham Biologicals s.a.), such combination vaccines being specially beneficial for vaccines for adolescents. Prefered combinations are based around a "core" combination of a Hepatitis B antigen (Hep B) and a Herpes Simplex (HSV) antigen. Optionally, to this "core" may be added one or more antigens derived from the following group: Eptein Barr Virus (EBV) antigen, Hepatitis A antigen (Hep A), Human Papilloma Virus (HPV) antigen. These combination vaccines may additionally comprise Varicella Zoster Virus (VZV), Human Cytomegalovirus (HCMV) or toxoplasma antigens.

Preferably the vaccine formulations of the present invention contain an antigen or antigenic composition capable of eliciting an immune response against a human pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat, nef, gp120 or gp160), human herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Rotavirus (including live-attenuated viruses), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpI, II and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen or a derivative thereof),

hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, and 18), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus, or derived from bacterial pathogens such as *Neisseria spp,* including *N. gonorrhea* and *N. meningitidis* (for example capsular polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *Streptococcus spp,* including *S. pneumoniae* (for example capsular polysaccharides and conjugates thereof, PsaA, PspA, streptolysin, choline-binding proteins), *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, lipoteichoic acids), *S. agalactiae, S. mutans; Haemophilus spp, including H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae* (for example OMP26, high molecular weight adhesins, P5, P6, lipoprotein D), *H. ducreyi; Moraxella spp, including M catarrhalis, also known as Branhamella* catarrhalis (for example high and low molecular weight adhesins and invasins*); Bordetella spp, including B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof, filamenteous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis and B. bronchiseptica; Mycobacterium spp., including M. tuberculosis* (for example ESAT6, Antigen 85A, -B or -C), *M. bovis, M. leprae, M. avium, M. paratuberculosis, M. smegmatis; Legionella spp,* including *L. pneumophila; Escherichia spp,* including *enterotoxic E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorragic *E. coli,* enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp,* including *V. cholera* (for example cholera toxin or derivatives thereof*); Shigella spp,* including *S. sonnei, S. dysenteriae, S. flexnerii; Yersinia spp,* including *Y.* enterocolitica (for example a Yop protein) , *Y. pestis, Y. pseudotuberculosis; Campylobacter spp,* including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli; Salmonella spp,* including *S. typhi, S. paratyphi, S. choleraesuis, S. enteritidis; Listeria spp.,* including *L. monocytogenes; Helicobacter spp,* including *H.* pylori (for example urease, catalase, vacuolating toxin); *Pseudomonas spp,* including *P.*

aeruginosa; Staphylococcus spp., including *S. aureus, S. epidermidis; Enterococcus spp.*, including *E. faecalis, E. faecium; Clostridium spp.*, including *C.* tetani (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus spp.*, including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium spp.*, including *C. diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia spp.*, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii; Ehrlichia spp.*, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp*, including *R. rickettsii; Chlamydia spp., including C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci; Leptospira spp.*, including *L. interrogans; Treponema spp.*, including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola, T. hyodysenteriae;* or derived from parasites such as *Plasmodium spp.*, including *P. falciparum; Toxoplasma spp.*, including *T. gondii (for example SAG2, SAG3, Tg34); Entamoeba spp.*, including *E. histolytica; Babesia spp.*, including *B. microti; Trypanosoma spp.*, including *T. cruzi; Giardia spp.*, including *G. lamblia; Leshmania spp.*, including *L. major; Pneumocystis spp.*, including *P. carinii; Trichomonas spp.*, including *T. vaginalis; Schisostoma spp.*, including *S. mansoni,* or derived from yeast such as *Candida spp.*, including *C. albicans; Cryptococcus spp.*, including *C. neoformans.*

In one preferred aspect the vaccine formulation of the invention comprises the HIV-1 antigen, gp120, especially when expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD2t as hereinabove defined.

In a preferred embodiment of the present invention vaccines containing the claimed adjuvant comprise the HPV viruses considered to be responsible for genital warts, (HPV 6 or HPV 11 and others), and the HPV viruses responsible for cervical cancer

(HPV16, HPV18 and others). Particularly preferred forms of vaccine comprise L1 particles or capsomers, and fusion proteins comprising one or more antigens selected from the HPV 6 and HPV 11 proteins E6, E7, L1, and L2. The most preferred forms of fusion protein are: L2E7 as disclosed in GB 95 15478.7, and proteinD(1/3)-E7 disclosed in GB 9717953.5.

Vaccines of the present invention further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum* include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P.falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. It's full structure is disclosed in the International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS,S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. faciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in Plasmodium spp.

The formulations may also contain an anti-tumour antigen and be useful for the immunotherapeutic treatment cancers. For example, the adjuvant formulation finds utility with tumour rejection antigens such as those for prostrate, breast, colorectal, lung, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, PRAME, BAGE or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical &

Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other Tumor-Specific antigens are suitable for use with adjuvant of the present invention and include, but are not restricted to Prostate specific antigen (PSA) or Her-2/neu, KSA (GA733), MUC-1 and carcinoembryonic antigen (CEA). Other antigens have been put forward as being pan-cancer therapeutic antigens including Tyrosinase and Survivin. Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen.

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from *Borrelia sp.*. For example, antigens may include nucleic acid, pathogen derived antigen or antigenic preparations, recombinantly produced protein or peptides, and chimeric fusion proteins. In particular the antigen is OspA. The OspA may be a full mature protein in a lipidated form virtue of the host cell (E.Coli) termed (Lipo-OspA) or a non-lipidated derivative. Such non-lipidated derivatives include the non-lipidated NS1-OspA fusion protein which has the first 81 N-terminal amino acids of the non-structural protein (NS1) of the influenza virus, and the complete OspA protein, and another, MDP-OspA is a non-lipidated form of OspA carrying 3 additional N-terminal amino acids.

Vaccines of the present invention may be used for the prophylaxis or therapy of allergy. Such vaccines would comprise allergen specific (for example Der p1, and pollen related antigens) and allergen non-specific antigens (for example the stanworth decapeptide).

The adjuvants of the present invention may also comprise further immunostimulants. One such example of an immunostimulant which has been described in combination with both alum and saponins is the bacteriallyderived monophosphoryl lipid A (MPL).

Monophosphoryl lipid A is a bacterially derived compound with adjuvant activity, and is a prefered immunostimulant for use in the present invention. This toxic compound has been altered to form less toxic derivatives, one such derivative is 3 De-O-acylated monophosphoryl lipid A (termed 3D-MPL or d3-MPL, to indicate that position 3 of the reducing end glucosamine is de-O-acylated). For preparation of 3D-MPL, see GB 2 220 211 A. Chemically it is a mixture of 3-deacylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. Preferably in the compositions of the present invention small particle MPL is used. Small particle MPL has a particle size such that it may be sterile-filtered through a 0.22μm filter. Such preparations are described in International Patent Application No. WO 94/21292. Further improvements are described in GB 9807933.8 which discloses stable preparations of 3D-MPL consisting of the tri and tetra acyl congeners.

Aluminium based vaccine formulations wherein the antigen and monophosphoryl lipid A (or derivatives thereof, such as 3-de-O-acylated monophosphoryl lipid A [3D-MPL]), are adsorbed onto the same particle are described in EP 0 576 478 B1, EP 0 689 454 B1, and EP 0 633 784 B1. In these cases then antigen is first adsorbed onto the aluminium salt followed by the adsorption of the immunostimulant 3D-MPL onto the same aluminium salt particles. Such processes first involve the suspension of 3D-MPL by sonication in a water bath until the particles reach a size of between 80 and 500 nm. The antigen is typically adsorbed onto aluminium salt for one hour at room temperature under agitation. The 3D-MPL suspension is then added to the adsorbed antigen and the formulation is incubated at room temperature for 1 hour, and then kept at 4°C until use.

Also desribed in WO 98/15287 are vaccine formulations comprising a metallic salt, a saponin, antigen, and the additional immunostimulant Monophosphoryl lipid A. In this document the saponin QS21 is associated with cholesterol containing liposomes, and this complex is adsorbed onto a complex of alum, antigen and 3D-MPL. Thus, the saponin, 3D-MPL and antigen are all adsorbed onto the same particle of metallic salt.

Thus, the present invention also takes the form of an adjuvant formulation comprising a combination of QS21 and 3D-MPL, adsorbed onto alum, characterised in that the alum particle is substantially free of other antigen. The 3D-MPL may be formulated together with the QS21 in the same liposomal structure, with the 3D-MPL and QS21 both associating within the bilayer. Alternatively the QS21 may be in the form of a liposome and the 3D-MPL may be in the form of a sub-220 nm particle, both formulations being adsorbed onto the same alum particle. A particularly prefered form of alum within the meaning of this formulation is aluminium hydroxide.

An additional alternative embodiment of the present invention is an adjuvant formulation comprising two major species of metallic salt particles: (a) a first metallic salt particle species which is adsorbed to a saponin, said metallic salt particle being substantially free of monophosphoryl lipid A, or derivative thereof; and (b) a metallic salt particle species which is adsorbed to monophosphoryl lipid A, or derivative thereof, said metallic salt particle being substantially free of saponin.

Also provided by the present invention is a vaccine comprising an adjuvant described in the paragraph above, and an additional complex comprising an antigen adsorbed onto a metallic salt particle.

Further provided is a process of producing a vaccine comprising the following steps:

(a) adsorbing a saponin and monophosphoryl lipid A, or derivatives thereof, to a metallic salt particle, in the absence of other antigen;

(b) adsorbing one or more antigen, or antigens, onto one or more metallic salt particle, or particles, in the absence of a saponin, or monophosphoryl lipid A, or derivatives thereof; and,

(c) admixing the products of steps (a) and (b).

The present invention further provides for the adjuvants and vaccines of the present invention for use in medicine, specifically as a method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy. Also provided for is the use of the adjuvants and vaccines of the present invention in the manufacture of a immunoprophylactic and immunotherapeutic treatment of viral, bacterial, parasitic infections, allergy, or cancer. The formulations of the present invention maybe used for both prophylactic and therapeutic purposes.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of antigen, preferably 1-500 μg, preferably 1-100 μg, most preferably 1 to 50 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced. Typically for human administration the saponin, including QS21, will be present in the range 1 μg - 1000 μg, preferably 10 μg - 500 μg, more preferably 20-200 μg per dose, more preferably 20-100μg per dose, and most preferably 10-50 μg per dose.

Vaccine preparation is generally described in "Vaccine Design - the subunit and adjuvant approach" Edited by Powell, M.F. and Newman, M.J.; 1995, Pharmaceutical Biotechnology (Plenum Press, New York and London, ISBN 0-306-44867-X)

The present invention is illustrated by, but not restricted to, the following examples.

Example 1, ***Materials and Methods***

Vaccine formulation

Vaccines and adjuvants of the present invention may be formulated according to the following procedure:

(I)Antigen (1-100 μg, preferably 10-50 μg) is added to alum (10-500 μg, most preferably 100 μg) and incubated for at least 1 hour. Adsorbtion of the antigen onto the alum is confirmed by centrifuging the sample and assaying the supernatant for antigen using ELISA or protein quantification.

(II)QS21 or other saponin is added to liposomes prepared containing a sterol such as cholesterol in the membrane. The liposomes are prepared by drying down a phospholipid such as dioleoyl phosphatidylcholine and cholesterol (4:1 w/w) from organic solvent and resuspending the lipids in water or buffer. The liposomes are then microfluidised or sonicated or extruded to generate small unilamellar vesicles. QS21 or other saponin is added to the liposomes so that the saponin:sterol ratio is between 1:5 and 5:1 (most preferably between 1:5 and 1:1). The saponin/liposome mixture is then added to alum. The optimal ratio for doing so is a sterol:alum ratio of 1:10 (w/w) under which conditions all of the liposomes bind to the alum. Since the saponin is associated with the cholesterol in the liposomes this results in quantitative binding of the saponin to the alum.

During the process of forming the liposomes, 3D-MPL or other lipid-A derivative can be dried down with the lipids so that it is incorporated in the resulting liposomes. The prefered ratio for doing so is to incorporate 3D-MPL at a ratio of MPL: cholesterol of 1:5 to 1:1. Then by following the above process a liposome-MPL/saponin mixture is added to alum resulting in adsorbtion of the sapinin and MPL to alum.

Alternatively 3D-MPL that has been suspended in water and microfluidised to achieve particle size of under 200 nm can be added to alum and then the liposome/saponin mixture added to alum. The preferred method for doing so is to add 50 μg of microfluised MPL to 400 μg alum resulting in quantitative binding of

the MPL, and then to add liposomes containing 50 μg cholesterol and to which 50 μg of QS21 have been added, resulting in quantitative binding of the liposome-saponin complex to the alum.

The vaccine is then formulated by taking the antigen-alum complex (I) and admixing it with the liposome/saponin/MPL-alum complex (II).

Claims

1. An adjuvant composition comprising a saponin, said saponin being adsorbed onto a metallic salt particle, characterised in that the metallic salt particle is substantially free of antigen.

2. An adjuvant composition composition as claimed in claim 1, wherein the metallic salt particle is a salt of aluminium, zinc, calcium, cerium, chromium, iron, or berilium.

3. An adjuvant composition composition as claimed in claims 1 or 2, wherein the metallic salt is a phosphate or hydroxide.

4. An adjuvant composition composition as claimed in any one of claims 1 to 3, wherein the metallic salt is aluminium hydroxide or aluminium phosphate.

5. An adjuvant composition composition as claimed in any one of claims 1 to 4, further comprising Monophosphoryl lipid A, or derivative thereof.

6. An adjuvant composition as claimed in claim 5, wherein the derivative of Monophosphoryl lipid A, is 3-O-deacylated monophosphoryl lipid A (3D-MPL).

7. An adjuvant composition composition as claimed in any one of claims 1 to 6, wherein the saponin is Quil A, or substantially pure fraction thereof.

8. An adjuvant composition according to claim 7, wherein the fraction of Quil A is QS21.

9. An adjuvant composition as claimed in claim 8, wherein the QS21 is formulated in a cholesterol containing vesicle.

10. An adjuvant composition as claimed in claim 5 or 6, wherein the saponin and monophosphoryl lipid A, or derivative thereof, are associated within a cholesterol containing vesicle.

11. An adjuvant composition as claimed in claim 5 or 6, wherein said saponin is associated with a cholesterol containing vesicle, and said monophosphoryl lipid A, or derivative thereof, is not associated with the said cholesterol containing vesicle.

12. A process for the manufacture of a vaccine composition comprising taking the adjuvant composition claimed in any one of claim 1 to 11, followed by the addition of antigen, characterised in that the antigen is adsorbed onto a metallic salt particle.

13. A process for the manufacture of a vaccine comprising the following steps: (1) adsorbing antigen to a first metallic salt particle; (2) adsorbing a saponin, or fraction thereof, to a second metallic salt particle; and (3) mixing the products of steps 1 and 2.

14. A process for the manufacture of a vaccine comprising the following steps: (1) adsorbing antigen to a first metallic salt particle; (2) adsorbing a saponin, and monophosphoryl lipid A, or fraction thereof, to a second metallic salt particle; and (3) mixing the products of steps 1 and 2.

15. A process as claimed in any one of claims 12 to 14, wherein the antigen or antigenic preparation is selected from the group comprising: Human Immunodeficiency Virus, Varicella Zoster virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Dengue virus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Hib, Meningitis virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma, stanworth decapeptide, Der p1, pollen related antigens; or Tumor associated antigens (TMA), MAGE, BAGE, GAGE, MUC-1, Her-2 neu, LnRH(GnRH), CEA, PSA, KSA, or PRAME.

16. A vaccine composition comprising two major populations of complexes, a first complex comprising (a) a saponin adsorbed onto a metallic salt particle, characterised in that said metallic salt particle is substantially free of antigen; and a second complex comprising (b) antigen adsorbed onto a metallic salt particle.

17. A vaccine composition comprising two major populations of complexes, a first complex comprising (a) a saponin adsorbed onto a metallic salt particle, characterised in that said metallic salt particle is substantially free of antigen; and a second complex comprising (b) antigen adsorbed onto a metallic salt particle, charcterised in that said metallic salt particle is substantially free of saponin.

18. A vaccine composition comprising two major populations of complexes, a first complex comprising (a) a saponin adsorbed and monophosphoryl lipid A, or derivative thereof, onto a metallic salt particle, characterised in that said metallic salt particle is substantially free of other antigen; and a second complex comprising (b) antigen adsorbed onto a metallic salt particle.

19. A vaccine composition comprising two major populations of complexes, a first complex comprising (a) a saponin and monophosphoryl lipid A, or derivative thereof, adsorbed onto a metallic salt particle, characterised in that said metallic salt particle is substantially free of other antigen; and a second complex comprising (b) antigen adsorbed onto a metallic salt particle, charcterised in that said metallic salt particle is substantially free of saponin and monophosphoryl lipid A, or derivative thereof.

20. A vaccine composition as claimed in any one of claims 16 to 19, wherein the metallic salt present in the first and second complexes are identical.

21. A vaccine composition as claimed in any one of claims 16 to 19, wherein the second complex comprises a plurality of sub-complexes, each sub-complex comprising a different antigen adsorbed onto a metallic particle.

22. A vaccine composition as claimed in any one of claims 16 to 21, wherein the metallic salt is a salt of aluminium, zinc, calcium, cerium, chromium, iron, or berilium.

23. A vaccine composition as claimed in claim 22 wherein the metallic salt is a phosphate or hydroxide.

24. A vaccine composition as claimed in claim 23 wherein the metallic salt is aluminium hydroxide or aluminium phosphate.

25. A vaccine composition as claimed in any one of claims 16 to 24, wherein the antigen is selected from the group comprising: Human Immunodeficiency Virus, Varicella Zoster virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Dengue virus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Hib, Meningitis virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma, stanworth decapeptide, Der p1, pollen related antigens; or cancer associated antigens, MAGE, BAGE, GAGE, MUC-1, Her-2 neu, LnRH(GnRH), CEA, PSA, tyrosinase, Survivin, KSA, or PRAME.

26. A vaccine composition as claimed in claim 25, wherein the antigen is a combination of Hepatitis A antigen and Hepatitis B antigen.

27. A vaccine composition as claimed in claim 25, wherein the plasmodium antigen is one or more antigens selected from the following group: RTS,S and TRAP.

28. A vaccine composition as claimed in claim 25 for use in medicine.

29. Use of vaccine composition as claimed in claim 25, for the manufacture of an immunotherapeutic treatment of viral, bacterial, parasitic infections, allergy, or cancer.

30. Use of an adjuvant composition as claimed in any one of claims 1 to 7, for the manufacture of an immunotherapeutic treatment of viral, bacterial, parasitic infections, allergy, or cancer.

31. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the administration of a safe and effective amount of a vaccine composition according to claim 25.